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STUDY OF THE BIOCIDAL PROPERTIES OF R_2O–RO–TiO_2–P_2O_5–R_2O_3–SiO_2 GLASS CERAMIC COATINGS

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It is found that the most informative and universal method of determining the biocidal properties of glass coatings is a quantitative method that takes account of the level of growth of biotest microorganisms seeded in liquid nutrients. It is shown that biocidality with respect to the bacteria *Escherichia coli* and the fungi *Candida albicans* of glass ceramic and composite glass coatings based on Na_2O–CaO–ZnO–TiO_2–Al_2O_3–P_2O_5–B_2O_3–SiO_2 glass is determined by the presence of calcium phosphates, zinc titanates, the bactericidal filler zinc phosphate in their structure. Depending on their inhibiting action biocidal glass ceramic and composite glass coatings can be used to protect different kinds of steel enamelware.

Key words: biocidal properties, calcium phosphates, zinc phosphates, glass ceramic coatings, microorganisms.

A global problem of modern times is securing a safe environment for society. The World Health Organization has asserted the need to study the effects of chemicals on living organisms, including humans. It is known that because of their chemical particulars heavy metals affect macro- and microorganisms and are antimicrobial agents having a denaturing effect and a high affinity to sulfur [1]. Heavy-metal ions act on strictly determined biochemical structures with a corresponding constitution by the receptor mechanism of interaction, which is the quickest mechanism and requires a lower dose of the substance. For this reason, heavy metals that are oligodynamic components initially block the active center of a biochemical structure and then bind the –SH groups of enzymes, which lose their force as a result. For example, mercury, which blocks the –SH groups in the protein structure of pale spirochetes, kills these microbes [2]. Heavy metals act in a similar manner on *E. Coli* bacteria [3] and other pathogenic microorganisms.

However, it is necessary to take into account the fact that heavy metals have not only a toxic effect on pathogenic microorganisms but also carcinogenic, mutagenic, and teratogenic effects on humans. Thus, As, Se, Zn, Cr, Be, Pb, Hg, Ag, and Ni at definite concentrations can give rise to malignant tumors, ZnS exhibits genotoxicity, and the metals Cd, Pb, Li, and Ga influence pathological changes in newborns. Some inorganic compounds, for example, chromium compounds, show allergenic effects in humans. It should be noted that under the combined action of a number of compounds, for example, Zn^{2+} and Cd^{2+}, synergism or potentiation, when the action of the elements is stronger than simple summation, is observed; conversely, when arsenic and selenium are used together this effect diminishes [1].

Chemical concentration determines the manifestation of heavy-metal toxicity in living organisms. Thus, Paracelsus, the founder of pharmaceutical chemistry, stated: "All things are poison, and nothing is without poison; only the dose permits something not to be poisonous." For this reason, heavy metals can be used as antimicrobial agents only when the concentrations at which they kill or delay the growth of pathogenic microorganisms fall within specific migration limits (SML) for a chemical substance in humans.

The most commonly used bactericidal components are cations of silver, zinc, titanium and other metals, for which the concentration affecting most pathogenic microorganisms falls within the SML for humans. Thus, with respect to the strength of their effect in humans zinc and titanium are class-3 hazards — substances which are moderately dangerous; their SML are 1.0 and 0.1 mg/liter [4, 5]. Titanium in concentrations 0.1 – 0.5 mg/liter stops bio-oxidation pro-

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cesses, but already at 5 mg/liter it has a cumulative toxic effect in humans and warm-blooded animals. Zinc in concentrations to 40 mg/liter in drinking water is harmless to humans, while in concentrations 1.4 – 2.3 mg/liter it is bactericidal with respect to *E. Coli* [1].

When using silver it is necessary to take account of the fact that this metal, which is a class-2 hazard, is characterized by high migratory power, its SML are 0.05 mg/liter [6]. At concentration 0.01 mg/liter, used for sterilizing water, silver is non-toxic to humans, does not inflame the membrane of the gastrointestinal tract, and does not change the taste of water. It is known that silver in concentrations 0.000001 – 0.5 mg/liter kills microorganisms and that at elevated concentrations in drinking water it is has a cumulative toxic effect in humans and warm-blooded animals. The fatal silver dose for humans is 10 g. Many silver salts in total amount 1 g cause argyria in humans — the skin, eyes, and mucous membranes become bluish-grey [1]. This fact shows that silver accumulates in the human body, indicating that silver must be monitored when used as a bactericidal agent.

Together with bactericidal activity another topical problem is fungicidal protection of objects used by humans. The significance of this problem is determined by the wide distribution in nature of pathogenic fungi, whose carriers are soil, plants, animals, insects, rotten produce, water, mold, blood, and canned foods. Pathogenic fungi are not nutrient demanding and unlike microbes withstand temperatures from 2 to 45°C.

One way to protect against pathogenic bacteria and fungi is to develop and adopt competitive, universal, protective-decorative glass coatings for medical, pharmaceutical, and household steel ware with biocidal functions.

We have confirmed that glass enamel coatings have promising applications for antibacterial protection of steel panels: a glass matrix with a programmed structure, bactericidal nanopowders of zinc phosphate, and composite glass coatings based on such powders has been obtained in the system R_2O–RO–TiO_2–P_2O_5–SiO_2. The composition of a coating with maximum bactericidal effect with respect to *E. Coli* has been optimized [7, 8].

The development of biocidal glass coatings requires, aside from synthesis of the compositions themselves, the development of a methodological approach to establishing their antibacterial and fungicidal functions and adapting to glass enamels the standards existing for these properties in other materials. The objective of the present work on the development of biocidal glass ceramic coatings was to develop a methodological approach and to synthesize coatings which are resistant to conventional pathogenic bacteria and microscopic fungi.

DEVELOPMENT OF A METHODOLOGICAL APPROACH

The methodological approach used to synthesize biocidal glass coatings envisages the development of a comprehensive procedure based on standard study methods [9, 10] for evaluating the phase composition, physical-chemical properties, and functional characteristics of glass enamels for wares as well as the antibacterial and fungicidal indicators for the coatings obtained.

The bacteria in the *E. Coli* group — *Escherichia coli* and the fungi in the *Candida* group — *Candida albicans*, which are conventional-pathogenic microorganisms, were chosen to study biocidal properties. This choice of biotests for glass coatings was based on the pathogenicity and wide distribution of these organisms in nature.

Escherichia coli are mobile, single-cell, gram-negative, rod-shaped bacteria, which do not form spores, and permanently inhabit the microflora in the lower intestine of humans and animals. The detection of these bacteria in food products and water and on equipment attests to fecal contamination, which is of great sanitary significance [3].

The yeast-like fungi *Candida albicans* — representatives of fungal microflora — are ubiquitous in the environment, live mainly in soil, are easily found on household objects, live on skin as saprophytes, mucous membranes, and urinogenital organs in humans, and often cause pathogenic micoses.

The bactericidal and fungicidal properties of glass coatings were determined by the following methods using solid and liquid culture media:

1st method — diffusion, or qualitative, based on the formation of a zone of growth retardation of the test microbe (*Escherichia coli* and *Candida albicans*) around the test sample (2 and 1.5 mm in diameter disks enameled on one side) [11, 12]; a solid agar-like culture medium is used; growth suppression on the section of contact between the object and the agar culture medium depends on the degree to which antimicrobial agents diffuse into the agar culture layer; this method is applicable only for migrating compounds;

2nd method — quantitative, based on the level of growth of the biotest microorganisms seeded in liquid culture media, in the presence and absence of test samples [13].

Selective culture media were used to obtain accumulating biotest cultures:

– 2% meat-extract agar (MEA) and beef-extract bullion (MEB) for culturing *E. coli* group bacteria;

– liquid and agar-like Czapek–Dox medium for culturing microscopic fungi with or without saccharose.

A bacterial culture in the exponential growth phase was used to prepare *Escherichia coli* unoculum — a suspension of cells, which is the start of a cell culture and used to seed a culture medium. For this, the daily culture of the strain grown on 2% MEA plates in test tubes at temperature 35 ± 2°C was washed off with sterilized tap water and the suspension of bacteria obtained was cultivated to concentration 10^6 – 10^7 microbial cells per 1 ml. The unoculum was standardized by direct count in a Goryaev chamber [11].

A *Candida albicans* unoculum was prepared by growing cultures of the fungi on agar-like selective Czapek–Dox me-

dia in test tubes at temperature 26 ± 2°C. To determine the fungicidal properties of the material with respect to the growing cells the fungi cultures were grown in 72 h to the exponential growth phase. At the end of this time, using a bacterial loop, the biomass of each type of fungus was transferred into sterilized tap water and a suspension of spores with the required concentration was obtained by serial culturing — $10^6 – 10^7$ microbe cells per 1 cm^3. The unoculum was standardized by direct count in a Goryaev chamber or by the turbidimetric method using a KFK-2 photoelectric colorimeter [11].

Each test sample, including the control sample, was placed in a test tube with the appropriate culture medium pre-seeded with the biotest suspension. All test tubes were hermetically sealed with cotton-gauze stoppers and arranged for incubation at room temperature with periodic shaking.

After exposure for 48 h, the cultures were sown "like a lawn" on MEA from the series of test tubes populated with *Escherichia coli*. For this, 0.5 ml of the suspension were taken from each test tube, placed on the MEA surface, and carefully spread with a spatula. The dishes were turned bottom up and incubated at 35 ± 2°C. After 24 h the dishes were examined and the growth of microcolonies was recorded.

The growth of fungal microflora in the test tubes was monitored 7 days after seeding and the active state of the microorganism was checked for vegetative forms of the fungi.

To obtain biocidal glass ceramic coatings, the cations Zn^{2+} and Ti^{4+} were chosen as the oligodynamic components.

The biocidal properties of the coatings being developed are obtained by directed crystallization during melting and heat-treatment of zinc- and titanium-containing glasses and glass ceramic coatings of a biocidal agent based on them — zinc titanate. Single-frit glass ceramic coatings obtained without introducing biocidal powders will make the product less expensive and more competitive.

EXPERIMENTAL PART

To achieve the objective indicated the system $Na_2O–CaO–ZnO–TiO_2–Al_2O_3–P_2O_5–B_2O_3–SiO_2$ was chosen for the glass matrix. The following were synthesized in the system studied:

12 compositions of titanium-containing calcium-zinc-silicophosphate glasses (ZP) in the high-silica region I with ZnO molar content from 2 to 12%;

4 compositions of titanium-containing zinc-silicophosphate glasses (ZP) in the low-silica region II with ZnO molar content from 12 to 22%.

The glasses were made at temperature 1250 – 1280°C followed by granulation in water.

A necessary condition for securing biocidal properties for the glass ceramic coatings during glassmaking and heat-treatment of the coatings based on the glasses is directed, volume, finely disperse, crystallization of hydroxyapatite (HAP), whose structure serves as a carrier of the biocidal metal cations, specifically, Zn^{2+}, and ensures that they are uniformly distributed in the surface layer of the coating. It is known that the zinc cation is adsorbed on the surface of HAP crystals and can partially, isomorphically replace Ca^{2+} in its structure [14]. Thus, pathogenic microorganisms, which survive by feeding on the macroelements calcium and phosphorus, simultaneously use the cation Zn^{2+} adsorbed on HAP, which retards their growth and, in consequence, intensifies the effect of the glass ceramic coatings.

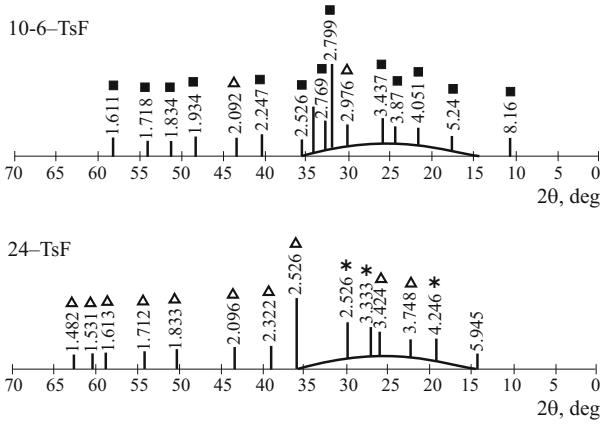

Fig. 1. Diffraction patterns of the experimental glasses after glassmaking: ∗) SiO_2 (quartz); ■) $Ca_5(PO_4)_3OH$; △) $Zn_2Ti_3O_8$.

RESULTS AND DISCUSSION

X-ray phase analysis (XPA) of the glasses after glassmaking and the coatings after heat-treatment was performed to study phase formation in the system studied.

XPA shows that a very small amount of zinc titanate crystallizes in the glasses in the high-silica region I as a result of the ratio ZnO/TiO_2 taking on values from 1 to 2.25 with heat-treatment during a short anneal for 3 – 5 min. When CaO is introduced into these glasses in amounts (molar content) from 4 to 11% and P_2O_5 from 4 to 5% with $CaO/P_2O_5 = 1.5 – 1.7$, finely disperse crystallization of HAP obtains. These glasses are characterized by a general decrease of the crystallization power of HAP after glassmaking as well as after heat-treatment with increasing ZnO content. The experimental glasses in the low-silica region II are characterized after glassmaking by a substantial quantity of zinc titanate, whose intensity in the glasses increases with heat-treatment with ZnO molar content in the glasses increasing from 12 to 22%.

To intensify the finely disperse volume crystallization of $Ca_5(PO_4)_3OH$ and $Zn_2Ti_3O_8$ the following were synthesized: invert low-alkali glasses 10-4–ZP, 10-5–ZP, and 10-6–ZP in the high-silica region I with $ZnO/TiO_2/CaO \approx 1$ and CaO molar content from 9 to 10% and TiO_2 from 8.5 to 9% and low-alkali 24–ZP in the low-silica region II with ZnO molar content 24% and $ZnO/TiO_2 = 3$. XPA showed that the 10-6–ZP and 24–ZP glasses are characterized by intense crystallization of zinc titanate, and a substantial amount of HAP is also observed in 10-6–ZP glass (see Fig. 1).

TABLE 1. Comparative Evaluation of the Fungicidal Properties of the Experimental Coatings and Monitoring of a Culture with respect to *Candida albicans*

Compositions of test samples, control comparison sample, and culture monitoring	Exposure start, 0 days		Exposure time, 7 days	
	Optical density D	Fungal cell concentration, cells/cm^3	Optical density D	Fungal cell concentration, cells/cm^3
10-6–ZP + $Zn_3(PO_4)_2$	0.40	2.35×10^6	0.10 (×10)	0.59×10^7
10-6–ZP	0.40	2.35×10^6	0.20 (×10)	1.17×10^7
24–ZP	0.40	2.35×10^6	0.14 (×10)	0.82×10^7
ÉSP-117	0.40	2.35×10^6	0.48 (×10^2)	2.83×10^8
C_{cult}	0.40	2.35×10^6	0.51 (×10^2)	3.10×10^8

The glasses 10-6–ZP and 24–ZP were chosen to study the biocidal properties. Glass ceramic coatings of the same kind were obtained on the basis of these glasses. To study the potentiation effect, bactericidal filler — zinc orthophosphate nanopowder in the amount 1.5 parts by weight per 100 parts glass by weight — was added to the 10-6–ZP glass.

The slip was deposited on 0.7 mm thick samples of low-carbon steel. The coatings were dried and fired at 780 – 800°C.

A comprehensive evaluation of the physical-chemical properties and operational characteristics of the 10-6–ZP and 24–ZP glass ceramic coatings and a composite glass coating based on 10-6–ZP showed that these coatings met the specifications for glass coatings used to protect enameled steel ware: chemical resistance — class A, luster — 55%, CLTE — 103×10^{-7} and 112×10^{-7} K^{-1}, respectively.

The titanium-containing enamel ÉSP-117 was chosen as the control sample for comparing the biocidal properties of the experimental coatings.

It was established on the basis of the studies of the inhibiting properties of the experimental coatings that their biocidal action in solid and liquid culture media is different.

Using the diffusion method it was established on the basis of the size of the growth-suppression zones around a disk and on the basis of the effectiveness of the biocidal properties [15] that the glass ceramic coating 24–ZP manifested biocidal action only with respect to the microscopic fungi *Candida albicans.* Examination of Petri dishes seeded with a *Candida albicans* culture with 10^7 cells/cm^3 showed a suppression zone around the 24–ZP sample. The same effect was also observed for an *Escherichia coli* culture as well as for the composite glass ceramic coating 10-6–ZP with $Zn_3(PO_4)_2$. The diameter of the suppression zone for these coatings was about 14 mm according to [11] or about 4 mm according to [12], i.e., a biocidal effect was observed.

Continuous "lawn-like" growth of colonies of microorganisms over the entire surface of the agar-like plates, including the space around all test samples, was observed on the agar plates of all Petri dishes seeded with cultures of the microorganisms *Escherichia coli* with 10-6–ZP, 24–ZP, and ÉSP-117 glass ceramic coatings and cultures of *Candida albicans* microorganisms with 10-6–ZP and 10-6–ZP + $Zn_3(PO_4)$ as well as ÉSP-117 coatings. Thus, no zones of suppressed growth of microorganisms were found.

Testing of the glass ceramic coatings for biocidal activity by the quantitative method in liquid media showed that the test samples 10-6–ZP, 10-6–ZP + $Zn_3(PO_4)_2$, and 24–ZP manifested suppression properties with respect to the *Escherichia coli* bacteria and the *Candida albicans* vegetative fungi cells.

After 7 days of exposure the concentration C_{cult} of the vegetative *Candida albicans* fungi cells in the culture medium in test tubes without the test samples increased 130-fold; this indicator increased 120-fold for ÉSP-117, 5-fold for 10-6–ZP, 2.5-fold for 10-6–ZP + $Zn_3(PO_4)_2$, and 3.5-fold for 24–ZP (see Table 1). The fungicidal properties of the experimental coatings and the checks of the culture with respect to *Candida albicans* are compared in Table 1.

Almost continuous growth of colonies covering nearly 100% of the surface was observed on an agar plate in a Petri dish where a test sample with ÉSP-117 with an *Escherichia coli* culture was seeded from a liquid medium after contact. In all other experimental variants the degree of microbial seeding was different and evaluated as a percentage of the microbial growth load of the control [13]. All glass ceramic coatings were characterized by an inadequate bactericidal effect: 15% for 10-6–ZP, 40% for 10-6–ZP + $Zn_3(PO_4)_2$, and 25% for 24–ZP.

These studies of the biocidal properties on solid and in liquid culture media have established that the composite glass ceramic coating 10-6–ZP + $Zn_3(PO_4)_2$ exhibits the strongest antibacterial and fungicidal effects as a result of potentiation with the simultaneous presence in it of the crystalline phases $Ca_5(PO_4)_3OH$, $Zn_2Ti_3O_8$, and the bactericidal filler $Zn_3(PO_4)_2$.

To establish the degree to which leaching of the zinc cation affects the biocidal properties of the experimental coatings, after the coatings were boiled for 48 h in distilled water the SML for Zn^{2+} in mg/liter was determined by the atomic-absorption method. This indicator was 0.028 mg/liter for the 10-6–ZP coating, 0.05 mg/liter for 10-6–ZP + $Zn_3(PO_4)_2$, and 0.319 mg/liter for 24–ZP and fell within the SML range for a component in humans.

In summary, this study of the phase composition, biocidal properties, and degree of leaching of zinc cations for the experimental glass ceramic and composite glass coatings established that the manifestation of their suppression properties on solid media depends on their crystallization power and the migration of Zn^{2+} cations. Thus, owing to the simultaneous presence of the crystalline phases of calcium phosphates and zinc titanates in the structure of the coating and zinc orthophosphate nanopowder introduced during milling, the composite glass ceramic coating 10-6–ZP + $Zn_3(PO_4)_2$ manifests a considerable bactericidal effect with respect to *Escherichia coli.* The manifestation of a fungicidal effect by the glass ceramic coating 24–ZP with respect to *Candida albicans* is due to the presence of a substantial quantity of zinc titanium. Quantitative tests of the experimental coatings 10-6–ZP, 24–ZP, and 10-6–ZP + $Zn_3(PO_4)_2$ for biocidal activity with respect to *Escherichia coli* and *Candida albicans* established that all coatings manifest suppression activity and that the strongest biocidal effect is found for the coating 10-6–ZP + $Zn_3(PO_4)_2$ because of potentiation.

CONCLUSIONS

It was established that the most informative and universal method of determining the biocidal properties of glass coatings is the quantitative method, which takes account of the level of growth of biotest microorganisms cultured in liquid media. It was shown that the biocidal action with respect to *Escherichia coli* bacteria and *Candida albicans* fungi of glass ceramic and composite glass coatings based on Na_2O–CaO–ZnO–TiO_2–Al_2O_3–P_2O_5–B_2O_3–SiO_2 is determined by the presence in their structure of calcium phosphates and zinc titanates as well as the bactericidal filler zinc phosphate. Depending on their suppression action the experimental biocidal glass ceramic and composite glass coatings can be used to protect enameled steel ware used for different purposes.

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